## REMARKS

Any fees that may be due in connection with this paper or with this application may be charged to Deposit Account No. 50-1213. If a Petition for extension of time is needed, this paper is to be considered such Petition.

Claims 26-29, 31, 32, 34-38, 40, 42, 44-46, 48-54, 57 and 65-91 are presently pending in this application.

Claims 35 is amended to more clearly point out and distinctly claim the subject matter by specifying that the chemokine receptor targeting agent binds to chemokine receptors. This does not add new matter nor change the scope of the claim, but merely emphasizes the nature of a chemokine receptor targeting agent subject matter of the claim. Claim 81 is amended to eliminate a duplicate claim. Claims 88-91, which find basis in the application as originally field, are added to claim alternative embodiments of independent claims. Therefore, no new matter is added.

An unexecuted DECLARATION pursuant to 37 C.F.R. §1.132 is provided with this response. The executed original will be provided upon receipt.

Marked-up claims pursuant to 37 C.F.R. §1.121 are attached.

## DECLARATION

A DECLARATION is provided to supplement the DECLARATION already of record. This DECLARATION and the prior DECLARATION of record provide data that show that the chemokine receptor targeting agent conjugates specifically target cells that express receptors to which the chemokine targeting agent binds and do so *in vivo* as well as *in vitro*. The DECLARATIONs provide data from a mouse xenograft model showing targeting of and cytotoxicity to activated, proliferating and migrating cells in tumors. The DECLARATIONs and discussion below evidence the nexus between *in vitro* data and *in vivo* efficacy and also address issues regarding the cited art.

**THE REJECTION OF CLAIMS 26-29, 31, 32, 34-38, 40, 42, 44-46, 48-54, 57 and 65-87 UNDER 35 U.S.C. §112, FIRST PARAGRAPH**

Claims 26-29, 31, 32, 34-38, 40, 42, 44-46, 48-54, 57 and 65-87 are rejected under 35 U.S.C. 112, first paragraph, because:

> the specification, while enabling for a method of producing cytotoxicity by contacting cells with a chemokine-toxin conjugate in vitro, allegedly does not reasonably provide enablement for a method of treating a pathological condition by administering a chemokine-toxin conjugate *in vivo*. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims.

The Examiner, citing In re Wands, 8 USPQ2d, 1400 (CAFC 1988) page 1404, concludes:

> Therefore, in summary, due to the lack of guidance and working examples of the use of chemokine-toxin conjugate to inhibit activation, proliferation, or migration of immune effector cells in vivo, as well as the unpredictability to one of ordinary skill in the art how use the chemokine-toxin conjugates to treat the claimed pathological conditions in vivo, especially given the prior teachings in the art, leads the Examiner to conclude that undue experimentation is necessary to practice the invention as claimed.

This rejection is respectfully traversed.

**RELEVANT LAW**

To satisfy the enablement requirement of 35 U.S.C § 112, first paragraph, the specification must teach one of skill in the art to make and use the invention without undue experimentation. *Atlas Powder Co. v. E.I. DuPont de Nemours*, 750 F.2d 1569, 224 USPQ 409 (1984). This requirement can be met by providing sufficient disclosure, either through illustrative examples or terminology, to teach one of skill in the art how to make and how to use the claimed subject matter without undue experimentation. This clause does not require "a specific example of everything *within the scope* of a broad claim." *In re Anderson*, 176 USPQ 331, at 333 (CCPA 1973), emphasis in original. Rather, the requirements of § 112, first paragraph "can be fulfilled by the use of

illustrative examples or by broad terminology." *In re Marzocchi et al.*, 469 USPQ 367 (CCPA 1971)(emphasis added).

Further, because "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." *In re Grimme, Keil and Schmitz*, 124 USPQ 449, 502 (CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. *Smith v. Snow*, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

Thus, there is no requirement for disclosure of every species within a genus. Applicant is entitled to claims are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

**PTO GUIDELINES**

The standard for determining whether the specification meets the enablement requirement is whether it enables any person skilled in the art to make and use the claimed subject matter without **undue** experimentation. *In re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400 (Fed. Cir. 1999) (emphasis added). In determining whether any experimentation is "undue," the above-noted factors are to be considered.

As instructed in the published PTO guidelines, it is improper to conclude that a disclosure is not enabling based on an analysis of only one of the above factors while ignoring one or more of the others. The analysis must consider all the evidence related to each of the factors, and any conclusion of non-enablement must be based on the evidence as a whole. *Id.* 8 USPQ2d at 1404 & 1407.

The starting point in an evaluation of whether the enablement requirement is satisfied is an analysis of each claim to determine its scope. As set forth in the guidelines, all questions of enablement are evaluated against **the claimed subject matter**. The focus of the inquiry is whether everything within the scope of the claim is enabled. With respect scope of enablement, the only relevant concern should be whether the scope of enablement provided to one skilled in the art by the disclosure is commensurate with the scope of protection sought by the claims. *In re Moore,* 439 F.2d 1232, 169 USPQ 236 (CCPA 1971). Once the scope of the claims is addressed, a determination must be made as to whether one skilled in the art is enabled to make and use the entire scope of the claimed invention without undue experimentation.

Before addressing issues raised by the Examiner, it is noted that the Examiner has made sweeping statements and conclusions without providing support therefor.

The Examiner is reminded that MPEP 2144.03 states:

> The Examiner may take official notice of facts outside of the record which are capable of instant and unquestionable demonstration as being "well-

> known" in the art. In re Ahlert, 424 F.2d 1088, 1091, 165 USPQ 418, 420 (CCPA 1970). . . ..

The scientific conclusions regarding what the skilled artisan knows and does not know that are set forth in the Office Action are"capable of instant and unquestionable demonstration as being "well-known" in the art. Evidence beyond official notice by the Examiner must be provided to establish that one of ordinary skill in the art would have been led to do what applicant has done:

> MPEP 2144.03 continues:
>
> If justified, the examiner should not be obliged to spend time to produce documentary proof. If the knowledge is of such notorious character that official notice can be taken, it is sufficient so to state. In re Malcolm, 129 F.2d 529, 54 USPQ 235 (CCPA 1942). If the applicant traverses such an assertion the examiner should cite a reference in support of his or her position.

In this instance, a reference or references supporting assertions made by the Examiner should be provided.

**Analysis**

As discussed in detail, below, the level of skill and knowledge of those who practice in this art, the guidance in the specification, the fact that the claims parallel the disclosures, and the nature of the experimentation, which is routine, as well as the evidence and information in the DECLARATION provided herewith and the DECLARATION of record in the application, lead to the conclusion that it would not require undue experimentation to practice the claimed methods.

**The rejected claims**

All of the rejected claims are directed to methods in which conjugates that contain a chemokine receptor targeting agent bind to chemokine receptors on targeted cells.

To focus of the remarks herein, subject matter of the claims, particularly the independent claims is summarized:

> Claim 29 is directed to methods of treating pathological conditions by:

treating the underlying pathology associated with inflammatory responses and secondary tissue damage associated with activation, proliferation and migration of immune effector cells by inhibiting activation, proliferation or migration of immune effector cells, by administering a conjugate to an animal whereby activation, proliferation, migration of the immune effector cells is inhibited.

The conjugate contains a chemokine receptor targeting agent or a portion thereof sufficient to bind to the chemokine receptor and facilitate internalization of the conjugate; the chemokine receptor targeting agent binds a receptor and internalizes the targeted agent in a cell to alter metabolism or gene expression in the cell, regulates or alters protein synthesis in the cell, inhibits proliferation of the cell or kills the cell.

Claim 35 is directed to methods of targeted delivery an agent into cells that express chemokine receptors by:

associating the agent with a chemokine receptor targeting agent, whereby:

the *chemokine receptor targeting agent binds to a chemokine receptor* expressed on the cells; and

the agent is internalized by the cells.

Claim 38 is directed to methods of inhibiting proliferation, migration or activation of cells bearing chemokine receptors by:

contacting the cells with an effective amount of a conjugate that comprises a targeted agent and a chemokine receptor targeting agent, or a portion thereof, wherein the *conjugate binds to a chemokine receptor* resulting in internalization of the targeted agent in cells bearing the receptor.

Claim 72 is directed to methods of inhibiting proliferation, migration or activation of immune cells by:

contacting immune cells with a conjugate that comprises a targeted agent or a portion thereof and a chemokine receptor targeting agent whereby activation, proliferation, migration of the immune cells is inhibited, wherein:

the targeted agent or portion thereof is a toxin;

the chemokine receptor targeting agent is a chemokine or a fragment of thereof that binds to a chemokine receptor and internalizes the targeted agent; and

*the conjugate binds to a chemokine receptor* resulting in internalization of the targeted agent in cells bearing the receptor.

Claim 86 is directed to methods for developing methods of treatment of inflammatory disorders:

> A method of treating a disease or disorder associated with an inflammatory response by:
>
> identifying immune cells that are activated in the disease or disorder;
>
> identifying chemokine receptors expressed on the cells;
>
> preparing a conjugate or plurality thereof containing toxin linked to a chemokine or a plurality of chemokines that specifically bind to the identified chemokine receptors and effect or facilitate internalization of the toxin into the cells; and
>
> contacting the immune cells with the conjugate or plurality thereof.

All claimed methods target conjugates to cells that express chemokine receptors. As discussed in the DECLARATION, chemokine receptors are expressed on a particular defined subset of cells, whose role in inflammatory responses and secondary tissue damage are known.

**Analysis**

The following discussion demonstrates that there is evidence of record that 1) demonstrates claimed methods in recognized *in vivo* and *in vitro* models; and 2) it would not require undue experimentation to practice the methods as claimed.

**1. Nexus between the data provided and pharmacological effectiveness**

The DECLARATIONs and application demonstrate that exemplary conjugates are targeted as described and claimed in this application predicted and are cytotoxic to the targeted cells. There is no basis upon which to conclude that any other conjugates prepared as described in the application would fail to exhibit the targeting and cytotoxicity.

As discussed below by reference to numerous publications by those of skill in this art, the *in vitro* tests and *in vivo* described in the instant application and DECLARATIONs have been recognized to establish that immunotoxins and cytotoxic conjugates possess pharmacological activity that supports a finding of practical utility. In *In re Hartop*, 311 F.2d 249, 135 USPQ 419, 426-7 (CCPA 1962), the Court held that, when one skilled in the art would accept a particular

test or experiment as being reasonably predictable that a tested invention would operate as alleged or have the therapeutic effect as alleged, the burden on behalf of an applicant had been satisfied. The Court went on to note that Congress has assigned the task of protecting the public from the advertising, use and sale of harmful drugs to the Food and Drug Administration (FDA), and the Federal Trade Commission, not the U.S. Patent and Trademark Office.

The instant specification and DECLARATIONs demonstrate using *in vitro* and *in vivo* models that the chemokine receptor targeting conjugates are toxic to cells that express receptors targeted by the selected chemokine receptor targeting. In addition, the specification and DECLARATIONs show that the conjugates are not toxic to cells that do not exhibit an upregulation of such receptors or that do not express such receptors.

As shown in the specification, and in the DECLARATIONs, three exemplary and different conjugates OPL98110 (an MCP-1-Shiga toxin conjugate), OPL98111 (an SDF-1$\beta$-shiga toxin conjugate), and OPL98112 (an eotaxin-shiga toxin conjugate) specifically target and are cytotoxic to cells that are known to bear chemokine receptors specifically recognized by the targeting agent in each conjugate. This cytotoxicity and specificity is demonstrated in recognized *in vitro* assays and also in two instances in mouse xenograft models.

As taught in the specification MCP-1 specifically binds to CCR2 receptors, which are present in the activated microglial cells in the CNS. Given its profile of cell and receptor selectivity OPL98110 (MCP-1, CCR2) is an appropriate chemokine-toxin conjugate for use in the nervous system. The result in the DECLARATION demonstrate that it targets cells of monocytic lineage including THP-1 leukemia cells, primary human peripheral blood mononuclear cells (PBMCs) and T-cells. Other experiments, as described in the DECLARATION, demonstrated that this conjugate targets cells of monocytic lineage (i.e. THP-1 cells which are microglia and MNP-like) as well as human peripheral blood monocytes and T-cells, but not primary human neurons or

U251 cells (a glioma of astrocytic lineage). OPL98110 was shown to bind to only to activated proliferating monocytes.

The over-expression of MCP-1 and target receptors have been observed in a wide range of cancers. For, example MCP-1 is responsible for the large leukocyte infiltrates seen in breast, lung and ovarian cancers. MCP-1 has been shown to play a direct role in tumor associated angiogenesis (a first for an $\alpha$-chemokine family member) and tumor progression. Consistent with this OPL98110 was found to be highly toxic to MCF-7 breast carcinoma cells in culture.

OPL98111 is conjugate that contains $\alpha$-chemokine SDF-1$\beta$, which only binds to CXCR4 receptors. As described in the DECLARATION, the chemokine-toxin OPL-98111 (SDF-1$\beta$) targets U251 (astrocytoma), HT-29 (human colon carcinoma), and THP-1 (monocytoid leukemia) cells in culture (Figure 1, attached hereto), as well as primary human monocytes, T-cells, and primary human neurons, which all are known to express the targeted receptor. Figure 1 in the DECLARATION shows the cytotoxic activity of OPL-98111 on target cancer cells in culture. The data show that only the activated population of isolated monocytes (*i.e*, those with upregulated CXCR4 expression) are targeted.

The activity of OPL98112 was also demonstrated in a mouse xenograft model of human colon carcinoma cells, which are known to express the targeted receptor. OPL-98111 retarded tumor growth relative to control animals. Figure 2 attached to the DECLARATION shows the effects of the conjugate on the tumors in the animals. The Figure shows that tumors from treated animals exhibited far less live tumor mass than untreated animals. In addition, the tumors in the untreated animals showed greater vascularization. Also, in the treated animals, the tumors contained abundant monocytic cells, which clear cellular debris.

OPL98112, a fusion of eoxtaxin and shiga toxin, was also tested in a mouse xenograft model and shown to be non-toxic. Activity to the targeted receptors was demonstrated in *in vitro* cytotoxicity assays, showing a dose response in proliferating monocytes.

Thus, there is data of record that shows that these conjugates function as described in the application. They specifically target activated cells of the immune system and other cells that are known to express the targeted receptors. They are well-tolerated in animal models and shown to have *in vivo* activity.

Other toxin conjugates and immunotoxins that exhibit similar (or less) potency and specificity are recognized by those of skill in this art to be useful in methods of treatment. These conjugates manifest *in vitro* activity comparable to or less than the instantly claimed . For example:

1. U.S. Patent No. 5,206,353 to Berger, *et al.* claims a portion of human CD4 containing a binding site for HIV gp120 linked to a portion of Pseudomonas exotoxin A essential for cell toxicity. The patent demonstrates usefulness of the protein using *in vitro* cytotoxicity data for the product encoded by CD4(178)-PE40 (a chimeric gene encoding the first 178 amino acids of CD4 and amino acids 1 to 3 and 253 to 613 of PE) on cells expressing HIV-1 envelope glycoprotein. Two test systems were employed. The first involved cells expressing the HIV envelope glycoprotein encoded by a recombinant vaccinia virus. FIG. 4a shows that protein synthesis in CV-1 cells infected with a recombinant vaccinia virus encoding gp160 was inhibited after 4 hours of exposure to CD4(178)-PE40 with an $ID_{50}$ of 27 ng/ml = 27 $\mu$M = 27 x $10^6$ pM).

The second test system employed cells, designated 8E5, chronically infected with HIV. 8E5 is a human T-cell line that contains a single integrated copy of the HIV-1 (LAV) genome, constitutively synthesizes HIV proteins, including gp120, form syncytia when mixed

with CD4-bearing cells, and produce budding particles. Addition of CD4(178)-PE40 to 8E5 cells led to inhibition of protein synthesis with an $ID_{50}$ of 100 ng/ml (100 x $10^6$ pM; determined at 17.5 hours after addition of toxin).

Berger *et al.* concludes that the *in vitro* test systems indicated that the indicated that the HIV-infected cells were highly sensitive to the hybrid toxin and states:

> the data presented herein clearly establish that a fusion protein CD4(178)-PE40 specifically and efficiently kills HIV-infected cells. This allows the use of this recombinant toxin as a therapeutic agent for the control and treatment of AIDS.

3. U.S. Patent No. 5,082,927 to Pastan *et al.* describes an IL-4-PE40 fusion protein that selectively kills cells bearing IL4 receptors as evidenced by *in vitro* studies. CT4R cells, a murine T cell line, were chosen since they are known to have relatively large number of IL4 receptors. This cell line can grow in the presence of either IL4 or IL2 and was maintained in culture medium containing recombinant murine IL4. The concentration of IL4-PE40 giving a 50% reduction of protein synthesis ($ID_{50}$) was about 17 ng/ml (17 x $10^6$ pM). To test cells of B-cell lineage and a murine myeloma P3X63-Ag8.653 was also tested. and found to be sensitive with $ID_{50}$ of about 12 ng/ml (12 x $10^6$ pM).

Pastan *et al.* concludes that the above *in vitro* data and other such *in vitro* data that establishes that IL4-PE40 is selectively toxic for IL-4-receptor bearing indicates that:

> the availability of IL4-PE40 now makes it possible to suppress immune response. It has been reported that activation of B and T cells with mitogen or anti-IgM antibody produces a 5- to 10-fold increase in IL4-receptor number (Park *et al.* (1987) *J. Exp. Med. 166*:476-488). Hence IL4-PE40 could be utilized for immuno-suppression by depleting activated lymphocytes. IL4-PE40 could also be used for the treatment of certain tumors because it has been

reported that certain tumor cell lines derived from B-lymphomas, T-Leukemias, mastocytomas and the like have relatively high number of IL4-receptors.

4. U.S. Patent No. 4,958,009 to Bjorn *et al.* broadly claims an immunoconjugate containing a cytotoxic agent and a monoclonal antibody and particularly describes and claims immunoconjugates of PE constructs and various monoclonal antibodies specific for ovarian tumors. The patent provides *in vitro* cytotoxicity data (reproduced below) against several ovarian cancer cell lines and cell lines that do not express the tumor antigens.

TABLE 13

$ID_{50}$-Values in ng/ml (nM) for Protein Synthesis Inhibition<a>

| Cells | 454C11-PE | 260F9-PE | 280D11-PE |
|---|---|---|---|
| OVCAR-2 | 1.6 (0.01) | 3.4 (0.02) | 835 (4) |
| OVCAR-3 | 3.6 (0.02) | 41.5 (0.2) | 805 (4) |
| OVCAR-4 | 0.7 (0.005) | 4.7 (0.02) | 54 (0.3) |
| OVCAR-5 | 10 (0.05) | 23 (0.1) | 3450 ( > 15) |
| A1847 | 2.5 (0.015) | 385<c> (2) | 2200 ( > 10) |
| KB | 15<b> (0.08) | > 600 ( > 3) | > 250 ( > 1) |

n<a> If not otherwise mentioned, these values are mean values of at least two experiments. -
n<b> Results from one experiment. -
n<c> Non-specific to toxicity. -

Some *in vivo* mice data is also provided in this patent, thereby evidencing that *in vitro* data can be extrapolated to *in vivo* effectiveness.

5. U.S. patent application Serial No. 07/278,601 (apparently available from NTIS; see, also Siegall *et al.* (1988) *Proc. Natl. Acad. Sci. USA 85*:9738-9742) describes and IL6-PE immunotoxin that, as evidence by *in vitro* cytotoxicity studies is selectively cytotoxic to IL-6 receptor-bearing cells, such as human myelomas.

The application concludes that this immunotoxin may be used may also be used in determining if cell lines express IL6 receptors (see, *e.g.*, page 9741 of Siegall *et al.*).

6. Siegall *et al.* (1989) *The FASEB J. 3*:2647-2652, describes cytotoxic activities of a TGF$\alpha$-PE hybrid protein and characterizes its cytotoxicity using *in vitro* assays using various cell lines that express EGF receptors (see, *e.g.*, Fig. 5, which demonstrates $ID_{50}$ values for protein synthesis inhibition in such cell lines are on the order of 0.5 ng/ml (0.5 $\mu$M = 0.5 x $10^6$ pM).

7. Siegall *et al.* (1991) *The FASEB J. 5*:2843-2849, describes cytotoxic activities of FGF-PE hybrid proteins and describes *in vitro* assays from which *in vivo* uses were deduced.

8. Biro *et al.* (1992) *Circ. Res. 71*:640-645, "In vitro effects of a recombinant toxin targeted to the fibroblast growth factor receptor on rat vascular smooth muscle and endothelial cells" describes an acidic FGF-PE conjugate and provides *in vitro* data showing that the conjugate inhibits smooth muscle cells. Biro *et al.* concludes that this data evidences use of the conjugate for treatment of restenosis.

9. Herschman *et al.* (1982) pages 381-95 in *Cold Spring Harbor Conf. Cell Proliferation*, Volume 9 Growth Cells Horm. Defined Media, Book A describes EGF-ricin conjugates and describes in detail a model system for testing the activity of such conjugates (see, *e.g.*, pages 383) and the results. Herschman *et al.* also indicates that the conjugates are useful for selecting cell lines and for *in vitro* killing of specific cells in order to identify cells that express or do not express particular antigens (see, *e.g.*, pages 392-393). Herschman *et al.* (which was published in 1982) concludes that:

> **it is likely that these reagents [cytotoxic conjugates and immunotoxins] will become important tools in the methodology of cell biology (page 393).**

Herschman *et al.*, thus, evidences that those of skill in this art, years before the filing date of the instant application, consider cytotoxic conjugates and immunotoxins are useful biological reagents.

10. Thorpe *et al.* (1985) *J. Natl. Cancer Inst. 75*:151-159 demonstrates that *in vitro* cytotoxic activity of immunotoxins are, although not proportionately related, correlated with *in vivo* cytotoxicity (see, *e.g.*, page 157). Thus, conjugates that exhibited anti-tumor activity *in vitro* exhibited such activity *in vivo*.

11. Other U.S. Patents that claim cytotoxic conjugates and immunotoxins and that teach that *in vitro* cytotoxicity studies are correlated with *in vivo* activity:

U.S. Patent No. 4,952,394;

U.S. Patent No. 4,962,188;

U.S. Patent No. 4,545,985;

U.S. Patent No. 4,925,922; and

numerous others.

**Xenograft model is recognized model to demonstrate therapeutic effectiveness**

The mice xenograft model is a recognized model that is used to assess the *in vivo* efficacy of antitumor agents. The following list (obtained from the Chemical Abstracts database available through dialog), which is by no means comprehensive, of publications that describe use of this model to extrapolate from effectiveness at reducing tumor size in nude mice to *in vivo* effectiveness in humans:

1. Chemotherapy of childhood rhabdomyosarcomas growing as xenografts in immune-deprived mice
AUTHOR(S): Houghton, Janet A.; Houghton, Peter J.; Green, Alexander A.
JOURNAL: Cancer Res. DATE: 1982 VOLUME: 42 NUMBER: 2 PAGES: 535-9

IDENTIFIERS: neoplasm inhibitor screening model mouse, child rhabdomyosarcoma antitumor screening model
DESCRIPTORS:
Mouse...immune-deprived, human tumor graft in, as antitumor screening model

2. Antitumor activities of seventeen alkylating agents against human mammary carcinoma (MX-1) in nude mice
AUTHOR(S): Inoue, Katsuhiro; Fujimoto, Shuichi; Ogawa, Makoto
JOURNAL: Nagoya J. Med. Sci. DATE: 1981 VOLUME: 43 NUMBER: 3-4
PAGES: 89-100
IDENTIFIERS: mammary carcinoma model antitumor agent, alkylating agent breast tumor xenograft
DESCRIPTORS:
Mouse,nude...
human mammary carcinoma xenograft in, as screening model for neoplasm inhibitors
Mammary gland,neoplasm, carcinoma...
neoplasm inhibitor screening model for
Transplant and Transplantation,animal, xeno-...
of mammary carcinoma in nude mice, as screening model for neoplasm inhibitors
Neoplasm inhibitors,carcinoma...
of mammary gland, screening model for

3. Chemotherapy responsiveness of human tumors as first transplant generation xenografts in the normal mouse: six-day subrenal capsule assay
AUTHOR(S): Bogden, Arthur E.; Cobb, William R.; Lepage, Doreen J.; Haskell, Paula M.; Gulkin, Theodore A.; Ward, Allen; Kelton, Diane E.; Esber, Henry J.
JOURNAL: Cancer (Philadelphia) DATE: 1981 VOLUME: 48 NUMBER: 1
PAGES: 10-20
IDENTIFIERS: cancer chemotherapeutic screening model, neoplasm inhibitors screening model, breast cancer treatment model
DESCRIPTORS:
Neoplasm inhibitors...
screening model for

4. Chemotherapy of human colorectal tumor xenografts in athymic mice with clinically active drugs: 5-fluorouracil and 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU). Comparison with doxorubicin derivatives: 4'-deoxydoxorubicin and 4'-O-methyldoxorubicin
AUTHOR(S): Giuliani, Fernando C.; Zirvi, Karimullah A.; Kaplan, Nathan O.; Goldin, Abraham
JOURNAL: Int. J. Cancer DATE: 1981 VOLUME: 27 NUMBER: 1 PAGES: 5-13
IDENTIFIERS: colorectal neoplasm fluorouracil BCNU, doxorubicin deriv colorectal neoplasm, screening model anticancer drug

5. Comparison of antitumor activities of nitrosourea derivatives against mammary breast carcinoma (MX-1) in nude mice
AUTHOR(S): Inoue, Katsuhiro; Fujimoto, Shuichi; Ogawa, Makoto
JOURNAL: Gann DATE: 1980 VOLUME: 71 NUMBER: 5 PAGES: 686-91
IDENTIFIERS: nitrosourea deriv antitumor, mouse nude antitumor drug
DESCRIPTORS:
Mouse,nude...
neoplasm inhibitors evaluation in, as animal model

Neoplasm inhibitors...
nitrosoureas as, in nude mouse-human tumor xenograft system

6. An experimental model of cachexia induced by a xenografted human tumor
AUTHOR(S): Strain, Alastair J.; Easty, Gerald C.; Neville, A. Munro
JOURNAL: JNCI, J. Natl. Cancer Inst. DATE: 1980 VOLUME: 64 NUMBER: 2
PAGES: 217-21
IDENTIFIERS: cachexia cancer body water, intestine absorption cachexia cancer, nutrition cachexia cancer
DESCRIPTORS:
Neoplasm-host relationship...
cachexia pathogenesis in mouse model
Cachexia...
in cancer, mouse model for, pathogenesis of

7. Chemotherapy studies with human colon cancer xenografts in nude mice
AUTHOR(S): Osieka, R.
JOURNAL: Curr. Chemother., Proc. Int. Congr. Chemother., 10th EDITOR: Siegenthaler, Walter (Ed), Luethy, Ruedi (Ed), DATE: 1978 VOLUME: 2,
PAGES: 1149-51 PUBLISHER: Am. Soc. Microbiol.,Washington, D. C
IDENTIFIERS: colon cancer neoplasm inhibitor, mouse neoplasm inhibitor screening, animal model colon cancer
DESCRIPTORS:
Cancer,colon... nude mouse as model for
Intestine,colon,neoplasm...
nude mouse as screening model for

8. Sensitivity of a human tumor xenograft in nude (athymic) mice to various clinically-active drugs
AUTHOR(S): Ovejera, Artemio A.; Houchens, David P.; Barker, Anna D.
JOURNAL: Proc. Int. Workshop Nude Mice DATE: 1977 VOLUME: 2, PAGES: 451-60
DESCRIPTORS:
Mouse,nude...
as animal model for cancer chemotherapy

9. Chemotherapy of human colon cancer xenografts in athymic nude mice
AUTHOR(S): Osieka, Rainhardt; Houchens, David P.; Goldin, Abraham; Johnson, Randall K.
JOURNAL: Cancer (Philadelphia) DATE: 1977 VOLUME: 40 NUMBER: 5, Suppl.
PAGES: 2640-50
DESCRIPTORS:
adenocarcinoma,neoplasm...
neoplasm inhibitors effect on, screening model for
Neoplasm inhibitors,carcinoma...
of colon, screening model for

10. Chemotherapeutic sensitivity to anticancer drugs of human tumor xenografts in athymic mice
AUTHOR(S): Ovejera, Artemio A.; Houchens, David P.; Barker, Anna D.
JOURNAL: Curr. Chemother., Proc. Int. Congr. Chemother., 10th EDITOR: Siegenthaler, Walter (Ed), Luethy, Ruedi (Ed), DATE: 1978 VOLUME: 2,
PAGES: 1144-6 CODEN: 37XLA2 LANGUAGE: English MEETING DATE: 77
PUBLISHER: Am. Soc. Microbiol.,Washington, D. C

IDENTIFIERS: neoplasm inhibitor evaluation nude mouse, animal model neoplasm inhibitor screening
DESCRIPTORS:
Neoplasm inhibitors...
evaluation of, nude mice as model for

11. Effect of serial passage in nude athymic mice on the growth characteristics and chemotherapy responsiveness of 13762 and R3230AC mammary tumor xenografts
AUTHOR(S): Bogden, Arthur E.; Kelton, Diane E.; Cobb, William R.; Gulkin, Theodore A.; Johnson, Randall K.
JOURNAL: Cancer Res. DATE: 1978 VOLUME: 38 NUM8ER: 1 PAGES: 59-64
IDENTIFIERS: neoplasm inhibitor screening model, mouse athymic neoplasm inhibitor screening
DESCRIPTORS:
Mouse,nude athymic...
as neoplasm inhibitor screening model, serial passage of tumor effecton
Neoplasm inhibitors...
screening of, nude athymic mouse as model for, serial passage of tumor effect on

12. Chemotherapy of human tumor xenografts in genetically athymic mice
AUTHOR(S): Ovejera, Artemio A.; Houchens, David P.; Barker, Anna D.
JOURNAL: Ann. Clin. Lab. Sci. DATE: 1978 VOLUME: 8 NUMBER: 1 PAGES:50-6
IDENTIFIERS: neoplasm inhibitor screening model, mouse athymic antitumor screening
DESCRIPTORS:
Mouse,athymic...
in neoplasm inhibitor screening

13. Comparative evaluation of the effectiveness of anticancer drugs against human lung cancer xenografts growing in mice in diffusion chambers
AUTHOR(S): Krutova, T. V.; Korman, D. 8.; Potapov, Yu. N.; Pashkova, V. S.
JOURNAL: Izv. Akad. Nauk SSSR, Ser. Biol. DATE: 1984 NUMBER: 6 PAGES:901-8 Neoplasm inhibitors...
screening of, human xenograft model for

14. Screening test of antitumor agents by human tumor cell lines in nude mice in ascitic form
AUTHOR(S): Kitahara, Takeshi; Minato, Keisuke; Shimoyama, Masanori
JOURNAL: Gan no Rinsho DATE: 1984 VOLUME: 30 NUMBER: 9 PAGES: 1158-67
DESCRIPTORS:
Mouse,nude...
model, for screening of antitumor agents

15. Characterization of a xenograft model of human ovarian carcinoma which produces ascites and intraabdominal carcinomatosis in mice
AUTHOR(S): Hamilton, Thomas C.; Young, Robert C.; Louie, Karen G.; 8ehrens, Brent C.; McKoy, Wilma M.; Grotzinger, Karen R.; Ozols, Robert F.
JOURNAL: Cancer Res. DATE: 1984 VOLUME: 44 NUMBER: 11 PAGES: 5286-90
DESCRIPTORS:
Antigens,CA 125...
expression of, by human ovarian carcinoma model in nude mice
Receptors...

for hormones, of human ovarian cancer model in nude mice
Ovary,carcinoma,neoplasm...
model of human, hormone receptors and growth characteristics of, in nude mice, treatment in relation to
Carcinoma...
model of ovarian human, hormone receptors and growth characteristics of, in nude mice, treatment in relation to
Transplant and Transplantation,animal...
of ovarian carcinoma of human, hormone receptors and growth characteristics of, in nude mice
Antigens... Estrogens... Hormones...
receptors for, of human ovarian carcinoma model in nude mice

16. Chemosensitivity of human gastrointestinal and breast cancer xenografts in nude mice and predictability to clinical response of anticancer agents
AUTHOR(S): Fujita, M.; Fujita, F.; Taguchi, T.
JOURNAL: Immune-Defic. Anim., Int. Workshop Immune-Defic. Anim. Exp. Res., 4th EDITOR: Sordat, Bernard (Ed), DATE: 1984 PAGES: 311-15 MEETING DATE: 820000 PUBLISHER: Karger, Basel, Switz
IDENTIFIERS: antitumor screening human xenograft mouse
DESCRIPTORS:
Digestive tract,neoplasm...
chemosensitivity of, in human xenograft-nude mouse model
Mouse,nude...
human tumors xenografted into, for antitumor screening

17. Growth pattern of tumor xenografts in Wistar rats after treatment with cyclophosphamide, total lymphoid irradiation and/or cyclosporin A
AUTHOR(S): Hoogenhout, J.; Kazem, I.; Jerusalem, C. R.; Bakkeren, J. A. J.; De Jong, J.; Kal, H. B.; Van Munster, P. J. J.
JOURNAL: Int. J. Radiat. Oncol., Biol. Phys. DATE: 1983 VOLUME: 9 NUMBER: 6 PAGES: 871-9
IDENTIFIERS: tumor xenograft growth rat model, immunosuppression tumor growth rat model
DESCRIPTORS:
Neoplasm inhibitors...
immunosuppressed rats with human and mouse xenografts for evaluation of
Radiotherapy...
immunosuppression from cyclophsophamide and cyclosporin A and, human and rat tumor xenograft growth response to, in rat model
Rat...
tumor xenografts of human and mouse in immunosuppressed, for neoplasm growth and neoplasm inhibitors evaluation

18. Human brain tumor xenografts in nude mice as a chemotherapy model
AUTHOR(S): Houchens, David P.; Ovejera, Artemio A.; Riblet, Sylva M.; Slagel, Donald E.
JOURNAL: Eur. J. Cancer Clin. Oncol. DATE: 1983 VOLUME: 19 NUMBER: 6 PAGES: 799-805
IDENTIFIERS: brain tumor xenograft chemotherapy model
DESCRIPTORS:
Neoplasm...
of brain of human, nude mouse xenograft of, as chemotherapy model

19. Evaluation of the response of a panel of human melanoma tissue-cultured cell lines xenografted in nude mice to four anticancer drugs of known clinical activity
AUTHOR(S): Bellet, Robert E.; Danna, Victoria; Mastrangelo, Michael J.; Eaton, Gordon J.; 8erd, David
JOURNAL: Proc. Int. Workshop Nude Mice DATE: 1982 VOLUME: 3rd NUMBER: Vol. 2 PAGES: 649-56
IDENTIFIERS: anticancer screening nude mouse model
DESCRIPTORS:
Mouse,nude...
human melanoma tissue-cultured cell line xenografted in, as anticancer screening model

20. New method for evaluating the effect of experimental chemotherapy on human xenografts in nude mice: use of lactate dehydrogenase isozyme
AUTHOR(S): Hayata, Satoshi; Fujita, Masahide; Nakano, Yosuke; Kumagai, Michihiko; Hakozaki, Michinori; Taguchi, Tetsuo
JOURNAL: Curr. Chemother. Immunother., Proc. Int. Congr. Chemother., 12th
EDITOR: Periti, Piero (Ed), Gialdroni Grassi, Giuliana (Ed), DATE: 1982
VOLUME: 2, PAGES: 1283-4 MEETING DATE: B10000 PUBLISHER: Am. Soc. Microbiol.,Washington, D. C
IDENTIFIERS: chemotherapy evaluation model, lactate dehydrogenase chemotherapy evaluation
DESCRIPTORS:
Neoplasm inhibitors...
model for evaluation of, neoplasms of humans xenografts in, lactate dehydrogenase detn. in relation to

21. Use of the nude mouse-human cancer xenograft system for testing sensitivity to anticancer drugs
AUTHOR(S): Fujita, Masahide; Taguchi, Tetsuo
JOURNAL: Gan to Kagaku Ryoho DATE: 1982 VOLUME: 9 NUM8ER: 4 PAGES: 606-15
IDENTIFIERS: antitumor drug screen graft model, mouse cancer graft antitumor screen
DESCRIPTORS:
Neoplasm...
grafts of human, in nude mouse, as system for neoplasm inhibitors screening

22. Combined modality treatment using radiation and/or chemotherapy in an athymic nude mouse-human medulloblastoma and glioblastoma xenograft model
AUTHOR(S): Slagel, Donald E.; Feola, Jose; Houchens, David P.; Ovejera, Artemio A.
JOURNAL: Cancer Res. DATE: 1982 VOLUME: 42 NUM8ER: 3 PAGES: 812-16
IDENTIFIERS: brain tumor chemotherapy radiotherapy, nude mouse brain tumor therapy
DESCRIPTORS:
Brain,neoplasm... Neoplasm...
chemotherapy and radiotherapy of, human tumors in nude mouse as model for

**23. The use of daunomycin-antibody immunoconjugates in managing soft tissue sarcomas: nude mouse xenograft model**
AUTHOR(S): Stastny, Jaroslav J.; Das Gupta, Tapas K.

JOURNAL: Cancer Res. DATE: 1993 VOLUME: 53 NUMBER: 23 PAGES: 5740-4
DESCRIPTORS:
Antibodies,conjugates...
daunomycin, fibrosarcoma of human inhibition by and tissue distribution of
Neoplasm inhibitors,fibrosarcoma...
daunomycin-antibody conjugates, of human cells
Pharmaceutical dosage forms,immunoconjugates...
of daunomycin, fibrosarcoma of human inhibition by and tissue distribution of

24. Comparison of the chemosensitivity between clinical specimens and their xenografts in nude mice by SDI test and the predictive value of in vivo chemosensitivity test using nude mice
AUTHOR(S): Sakamoto, Yasuo; Fujita, Masahide; Goi, Mamiyo; Tsukamoto, Fumine; Sugimoto, Takuji; Okuyama, Yasushi; Rhi, Minehide; Kusuyama, Takatsugu; Fujita, Fumiko
JOURNAL: Gan to Kagaku Ryoho DATE: 1993 VOLUME: 20 NUMBER: 4 PAGES: 447-54 CODEN: GTKRDX ISSN: 0385-0684 LANGUAGE: Japanese
IDENTIFIERS: antitumor chemosensitivity succinate dehydrogenase inhibition
DESCRIPTORS:
Neoplasm inhibitors...
chemosensitivity test for, using succinate dehydrogenase inhibition, in clin. specimens and nude mouse model
9002-02-2 anticancer drugs chemosensitivity test using inhibition of, in clin. specimens and nude mouse model

25. Evaluation of metastatic human tumor burden and response to therapy in a nude mouse xenograft model using a molecular probe for repetitive human DNA sequences
AUTHOR(S): Shoemaker, Robert H.; Smythe, Anne M.; Wu, Lin; Balaschak, Michael S.; Boyd, Michael R.
JOURNAL: Cancer Res. DATE: 1992 VOLUME: 52 NUMBER: 10 PAGES: 2791-6
IDENTIFIERS: nude mouse model human tumor metastasis, hybridization repetitive DNA probe
DESCRIPTORS:
Nucleic acid hybridization,DNA-DNA, dot-blot...
human repetitive DNA probes in, for anal. of human tumor metastasis in nude mice models

26. Toxicity of anticancer agents, growth and chemosensitivity of human tumor xenografts in a segregating stock of AF nude mice
AUTHOR(S): Maruo, Kohji; Emura, Reiko; Ohnishi, Yasuyuki; Endo, Sachio; Ueyama, Yoshito; Nomura, Tatsuji
JOURNAL: Lab. Anim. DATE: 1991 VOLUME: 25 NUMBER: 4 PAGES: 342-7
IDENTIFIERS: antitumor screening mouse model

The above sampling of publications, all published years before the effective filing date of the instant application, clearly establishes that the rodent xenograft model is a recognized model for predicting the efficacy of antitumor

agents and certainly in establishing a pharmacological utility that is of benefit to the public and is practical. As stated above, in *Nelson v. Bowley*, the Court of Customs and Patent Appeals held that tests establishing pharmacological activity, such as the stimulation of smooth muscle tissue from gerbil colons and the modulation of the blood pressure in rats, manifest a practical utility. 206 USPQ 881 (CCPA 1980); *In re Bundy* 209 USPQ 48 (CCPA 1981). Therefore, the results set forth in the DECLARATION clearly establish a pharmacological effectiveness sufficient to meet the requisites of 35 U.S.C. §112, first paragraph.

**Other agents that target immune cells are therapeutically effective**

In addition, as described in the attached DECLARATION, Bexxar and Genimmune, which evidence that agents that are cytotoxic to immune cells, have *in vivo* activity, have demonstrated efficacy in human clinical trials, (the former to Phase III), and thus have shown efficacy not only in animal models but also in humans. Bexxar is awaiting FDA approval.

Two other fusion proteins Zevalin for lymphoma (from IDEC Pharmaceuticals) and Myelotarg (AHP) for leukemia have been approved by the FDA. Ontak the Il-2 fusion, from Ligand Pharmaceuticals targets T-cells, is (FDA) approved for Lymphoma and is in Phase II trials for the T-cell mediated condition of Psoriasis. Novantrone is a DNA intercalating chemical that has FDA approval for the treatment of progressive multiple sclerosis. This agent is essentially an anticancer drug and is cytocidal to proliferating macrophages and T-cells which are the recognized cells underlying the pathology of the disease (insert sheet from www.amgen.com). Colchicine is a plant derivative approved by the FDA for gout and arthritic gout. The anti-inflammatory effects of this molecule are thought to be achieved by inhibiting the proliferation of immune cells by binding to intracellular microtubules that are essential for meiosis and mitosis. Many antibodies are under preclinical and clinical development for a wide array of immune cell mediated diseases. For example, anti-CD147

monoclonal antibody (ABX-CBL) from Abgenix (www.abgenix.com) has completed a Phase II trial for acute graft-versus-host disease. The antibody kills different groups of immune cells through complement-mediated lysis.

These examples (and there are many more) demonstrate that elimination of immune cells in various diseases is a valid approach to therapy. They approach the elimination by different means and, thus, do not teach the eradication of such cells by using agents that exploit chemokine receptors or their ligands as claimed in this application.

As stated in the DECLARATION, the beauty of the chemokine system that the instant application and instantly claimed methods exploit, is that there is a greater deal of targeting specificity and versatility. For example CCR3 expressing TH2 cells and eosinophils are implicated in the pathology of allergic asthma. The fusion with the eotaxin ligand (OPL98112) can eliminate both types of pathological cells; whereas an antibody can only eliminate on type or the other. OPL98112 exhibits no gross toxicity *in vivo* using normal animals (mentioned above).

**Conclusion**

The above patents and publications and other data provide evidence that a showing of *in vitro* cytotoxic activity is recognized by those of skill in this art to evidence sufficient pharmacological activity to support a finding that these conjugates will have therapeutic efficacy for the of immunotoxins and cytotoxic conjugates for treatment. These patents and publications evidence that those of skill in this art recognize that cytotoxic conjugates are therapeutically effective (see, *e.g.*, Herschman *et al.* (1982)) based upon data comparable to that provided in the applciatio. Each of the patents and publications provides a cytotoxic or immunotoxic conjugate and *in vitro* cytotoxicity studies and proposes *in vivo* and *in vitro* uses for the conjugates. In some instances, *in vitro* tests were subsequently followed by *in vivo* tests that supported the

conclusions from the *in vitro* tests (see, *e.g.*, U.S. Patent No. 4,958,009 to Bjorn *et al.*)

Since the instantly claimed conjugates process activity in similar *in vitro* and *in vivo* assays, those of skill in the art would, in light of the above patents and publications and numerous others, recognize that the *in vitro* data provided in this application provides sufficient evidence to support a conclusion that the conjugates are useful in methods of treatment as claimed. Those of skill in this art certainly would recognize that the instant application provides sufficient evidence to conclude that chemokine-receptor targetiong conjugates possess specificity for and activity against target chemokine-receptor bearing cells and that these conjugates will function *in vivo* as claimed and as described in the instant application.

Furthermore, as described in the DECLARATION and the application, the chemokine system is ideal for exploitation in this manner. There are a variety of different chemokine receptors and chemokine receptor targeting agents. Chemokine receptors are elevated in a variety of pathologies and are elevated in patterns characteristic of a particular disease and in a temporal manner in a disease. As a result, chemokine receptor targeting agents conjugates can be prepared as taught in the application by selecting a targeting agent that targets receptors on cells that are activated, migrate or proliferate in a particular disease. The application details how such selection is to be made. The data in the application and DECLARATIONs shows that the conjugates target the selected receptors and are cytotoxic to the targeted cells in recognized *in vitro* and *in vivo* models. This concept and claimed methods are generically described and claimed. As noted above, "it is manifestly impracticable for an applicant who discloses a generic invention to give an example of every species falling within it, or even to name every such species, it is sufficient if the disclosure teaches those skilled in the art what the invention is and how to practice it." *In re Grimme, Keil and Schmitz*, 124 USPQ 449, 502

(CCPA 1960). Thus, there is no doubt that a patentee's invention may be broader than the particular embodiment shown in the specification. A patentee not only is entitled to narrow claims particularly directed to the preferred embodiment, but also to broad claims that define the invention without a reference to specific instrumentalities. *Smith v. Snow*, 294 U.S. 1, 11, 24 USPQ 26, 30 (1935).

There is no requirement for disclosure of every species within a genus. Applicant is entitled to claims are commensurate in scope not only with what applicant has specifically exemplified, but commensurate in scope with that which one of skill in the art could obtain by virtue of that which the applicant has disclosed.

In this instance, the application discloses and claims a generic treatment modality; the applicant has disclosed as "a generic invention" and has provided persuasive evidence that it functions as claimed. Therefore, applicant has fulfilled the requirements of § 112, first paragraph "by broad terminology." *In re Marzocchi et al.*, 469 USPQ 367 (CCPA 1971)(emphasis added), and as discussed below, numerous detailed examples.

**2. It would not require undue experimentation to practice the claimed methods**

The inquiry with respect to scope of enablement under 35 U.S.C. §112, first paragraph, is whether it would require undue experimentation to make and use the claimed invention. A considerable amount of experimentation is permissible, particularly if it is routine experimentation. The amount of experimentation that is permissible depends upon a number of factors, which include: the quantity of experimentation necessary, the amount of direction or guidance presented, the presence or absence of working examples, the nature of the invention, the state of the prior art, the relative skill of those in the art, the predictability of the art, and the breadth of the claims. *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986); see also *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988).

**(A) The breadth of the claims**

As discussed above, all of the rejected claims are directed to methods in which conjugates that contain a chemokine receptor targeting agent bind to chemokine receptors on targeted cells to internalize a linked agent, such as a cytotoxin.

As discussed below and above, the application broadly and in great detail describes how to select chemokine receptor targeting agents and how to prepare targeting agent conjugates and how to use them therapeutically. Thus, the claims are of the same scope as the disclosure.

**(B) The relative skill of those in the art**

The level of this of those in this art is recognized to be high (see, , *Ex parte Forman*, 230 USPQ 546 (Bd. Pat. App. & Int'f 1986). Also, the prior patents and publications in this art (see, e.*g.*, those listed above, and those of record in this application), which are directed to those of skill in this art, are authored by those with advanced degrees and require a high level of skill and education to comprehend. Such publications, thus, evidence the high level of skill of those who work in this art.

**(C) Teachings in the specification**

As described in the application in great detail and summarized in the previous responses and discussed in the DECLARATIONs, the activation, migration and proliferation of leukocytes are the hallmark of a vast number of immunomodulatory diseases. These cells are responsible for the production of inflammatory mediators and toxic molecules (such as cytokines, reactive oxygen species, metalloproteinases and cytotoxins) that are essential for the host immune defense against invading pathogens, such as bacteria and viruses. Inappropriate triggering, disregulation or over-activation of the immune response is responsible for the damage to normal host tissue witnessed in leukocyte-mediated diseases such as arthritis, multiple sclerosis, and pulmonary diseases. Leukocyte-mediated diseases also include trauma (e.g. spinal cord injury) and

cancers and others. In the latter, leukocytes exert tumorigenic effects by nourishing the cancer directly or indirectly (by directing angiogenesis), by supplying chemokines and growth factors, and aiding metastasis by supplying various extracellular proteases.

Thus leukocytes are the mediators of diseases that can have combinations of allergic, autoimmune, angiogenic, inflammatory, and tumorigenic components. It must be noted that leukocytes are not necessarily the trigger of disease (which may be viral , bacterial, allergen, aberrant gene expression, trauma etc – initiated) but the excess immune (leukocyte) response is responsible for disease manifestation and progression.

This application provides an avenue of the therapeutic intervention that exploits this common underlying response (termed an underlying pathological response in the claims). Selection of this pathway for therapeutic intervention is not new (see discussion below); what is new in this application is the mode of intervention. The instant application provides conjugates that are targeted to specific chemokine receptors. (see, *e.g.*, Arimilli *et al.* (2000) *Immunological Rev. 177*:43-51).

The instant inventors recognized that chemokines play an intimate role in these varied diseases, and, as described in the application, provide a large repertoire of molecules that interact with an array of receptors. It is the instant inventors who have identified chemokine receptors as ideal targets for delivery of therapeutics, such as toxins.

Having provided the mode of intervention, the use of chemokines as targeting agents as described herein, one of skill in the art will recognize by virtue of knowledge in the art and the disclosure in the application, that the method provides a means for treatment of any disease in which in inappropriate triggering, disregulation or over-activation of the immune response is involved.

**(D) The amount of direction or guidance presented, the presence of working examples, the nature of the invention, and predictability**

The instant specification exemplifies and provides detailed instructions regarding how to make and use chemokine receptor targeting agent conjugates. The specification details a variety of diseases and identifies the receptors to target in each disease and also identifies agents that target the receptors.

The application exemplifies and details construction of 12 conjugates (see, *e.g.*, Table 6 and accompanying text and Examples). The specfication teach (and the DECLARATIONs also describe) how to make the conjugates, and, as how to select chemokine receptor targeting agents and details the specificity of a dozen or more exemplary conjugates. The specification also provides details of *in vitro* and *in vivo* models to test conjugates (see, page 110 *et seq.*, which provides details of models for a variety of diseases) and teaches how to formulate the conjugates for administration (page 132 *et seq.*).

The DECLARATIONs demonstrate that exemplary conjugates possess specificity and cytoxicity for targeted cells *in vitro* and *in vivo*, and do not exhibit toxicity *in vivo*. The previous DECLARATION and the application establish that the diseases are associated with activation, proliferation and migration of immune effector cells, including secondary tissue damage-promoting cells, and, thus, share a common underlying pathology. This coupled with the data in the DECLARATIONs, the detailed description in the application regarding specificity of a large number of chemokine targeting agents and exemplification of a dozen different conjugates with a description of each conjugate's specificity, provides more than adequate guidance to practice the claimed methods.

It would only require routine experimentation to prepare conjugates as described in the application and use them in the claimed methods. The data provided and description demonstrate the each conjugates targets the intended cells and is cytotoxic. Hence there is no issue of unpredictability.

**(E) The state of the prior art**

Also, as evidenced and discussed above, although the instant inventors are the first to exploit the chemokine system in this manner, the art is replete with examples of successful uses of cytotoxic conjugates and immunotoxins. The instantly claimed conjugates exhibit cytotoxicity and targeting specificity comparable to or greater than other cytotoxic conjugates and immunotoxins, and, thus there is no reason to doubt that they will possess *in vivo* activity.

**Conclusion**

Therefore, in light of the fact that the claims are tailored to the scope of the disclosure, the high level of skill in the art, the knowledge and extent of the prior art, the instant disclosure, which provides detailed guidance for selecting chemokine receptor targeting agents and numerous examples of conjugates, it would not require undue experimentation to practice the claimed methods.

**Rebuttal to the Examiner's comments**

1) The Examiner urges:

> First, Applicants have only provided guidance and working examples of the in vitro bioactivity of one chemokine-toxin fusion protein using an RIP assay (Example 2, page 166 of the specification). While this conjugate did inhibit protein synthesis in vitro, Applicants have not demonstrated that this, or any chemokine-toxin conjugate, is effective in inhibiting activation, proliferation, or migration of immune effector cells in vivo. No nexus has been presented in the specification or claims as to how the inhibition of protein synthesis by inhibiting translation of luciferase RNA can be predictable of in vivo effects on a T-cell population, especially given the teaching of Volk et al. that an in vivo response to a cytokine-toxin conjugate is unpredictable based on in vitro results (page 2504, first full paragraph). Volk et al. state that even though IL-2-PE40 improve the immunosupressive efficacy of the cell-mediated immune response, there is still an undesirable humoral response. Since chemokines are considered cytokines, the same unpredictability as to the in vivo response of a chemokine-toxin conjugate based on in vitro results would be expected. Therefore, it would not be predictable to one of ordinary skill in the art how use the chemokine-toxin conjugates to treat the claimed pathological conditions in vivo.

As discussed above, the specification provides numerous examples of chemokine receptor targeting agents and their specificities; provides *in vitro* and *in vivo* data for at least three different conjugates; and establishes that such data is recognized by those of skill in the art to evidence therapeutic effectiveness. The instant application provides a broad therapeutic modality, whose effectiveness is demonstrated by the disclosure and data in the application and data and discussion in the DECLARATIONs.

As described in the application in great detail and summarized in the previous responses, the activation, migration and proliferation of leukocytes are the hallmark of a vast number of immunomodulatory diseases. These cells are responsible for the production of inflammatory mediators and toxic molecules (such as cytokines, reactive oxygen species, metalloproteinases and cytotoxins) that are essential for the host immune defense against invading pathogens, such as bacteria and viruses. Inappropriate triggering, disregulation or over-activation of the immune response is responsible for the damage to normal host tissue witnessed in leukocyte-mediated diseases such as arthritis, multiple sclerosis, and pulmonary diseases. Leukocyte-mediated diseases also include trauma (e.g. spinal cord injury) and cancers and others. In the latter, leukocytes exert tumorigenic effects by nourishing the cancer directly or indirectly (by directing angiogenesis), by supplying chemokines and growth factors, and aiding metastasis by supplying various extracellular proteases.

Thus leukocytes are the mediators of diseases that can have combinations of allergic, autoimmune, angiogenic, inflammatory, and tumorigenic components. It must be noted that leukocytes are not necessarily the trigger of disease (which may be viral , bacterial, allergen, aberrant gene expression, trauma etc – initiated) but the excess immune (leukocyte) response is responsible for disease manifestation and progression.

This application provides an avenue of the therapeutic intervention that exploits this common underlying response (termed an underlying pathological response in the claims). Selection of this pathway for therapeutic intervention is not new ; what is new in this application is the mode of intervention. The instant application provides conjugates that are targeted to specific chemokine receptors.

The instant inventors recognized that chemokines play an intimate role in these varied diseases, and, as described in the application, provide a large repertoire of molecules that interact with an array of receptors. It is the instant inventors who have identified chemokine receptors as ideal targets for delivery of therapeutics, such as toxins.

Having provided the mode of intervention, the use of chemokines as targeting agents as described herein, one of skill in the art will recognize by virtue of knowledge in the art and the disclosure in the application, that the method provides a means for treatment of any disease in which in inappropriate triggering, disregulation or over-activation of the immune response is involved.

2. **The Examiner states:**

> In addition, Applicants have brought to the Examiner's attention on page 10 of the response dated 1/23/02 that the arguments regarding "Bexxar" and "Genimmune" (discussed on page 15 (Table 3) of Applicants' response dated 6/25/01) are to demonstrate operativeness and to evidence confirmation of what is taught in the instant specification. However, as stated by Applicants, these conjugates have not received FDA approval and, therefore, the effectiveness of these compounds and their use in treating immune diseases has not yet been established.

This is incorrect. As discussed above and in the DECLARATION Bexxar is awaiting FDA approval and both Bexxar and Genimmune have demonstrated *in vivo* efficacy. Further, as described in the DECLARATION because the instant conjugates target a different array of receptors from those that employ "classic" cytokines, which also induce humoral response, the instantly claimed conjugates will be less toxic (as evidenced by the *in vivo* animal model data). In

addition, as discussed in the DECLARATION, there are other examples of conjugates and immune-targeting agents that have been FDA approved. FDA approval is not the standard for a finding of utility under 35 U.S.C. §112, first paragraph. As noted, In *In re Hartop*, 311 F.2d 249, 135 USPQ 419, 426-7 (CCPA 1962), the Court held that, when one skilled in the art would accept a particular test or experiment as being reasonably predictable that a tested invention would operate as alleged or have the utility alleged, the burden on behalf of an applicant to has been satisfied. The Court stated that Congress has assigned the task of protecting the public from the advertising, use and sale of harmful drugs to the Food and Drug Administration (FDA), and the Federal Trade Commission, not the U.S. Patent and Trademark Office.

**THE REJECTION OF CLAIMS 35, 36, 38, 72-80, 84, 86 AND 87 UNDER 35 U.S.C. §102(b)**

Claims are 35, 36, 38, 72-80, 84, 86 and 87 are rejected under 35 U.S.C. §102(b) as being anticipated by Volk *et al.* (1994) because Volk *et al.* allegedly discloses a method of targeted deliver of an agent into cells that express chemokine receptors by associating the agent with a targeting agent, where the cells are activated leukocytes. The Examiner alleges that the claims "read on administration of chemokine-toxin conjugates because chemokines are members of the cytokine family and Il-2 receptors are expressed on immune cells." This rejection is respectfully traversed.

**Relevant law**

Anticipation requires the disclosure in a single prior art reference of each element of the claim under consideration. *In re Spada*, 15 USPQ2d 1655 (Fed. Cir, 1990), *In re Bond*, 15 USPQ 1566 (Fed. Cir. 1990), *Soundscriber Corp. v. U.S.*, 360 F.2d 954, 148 USPQ 298, 301, adopted 149 USPQ 640 (Ct. Cl.) 1966. See, also, *Richardson v. Suzuki Motor Co.*, 868 F.2d 1226, 1236, 9 USPQ2d 1913,1920 (Fed. Cir.), *cert. denied*, 110 S.Ct. 154 (1989). "[A]ll limitations in the claims must be found in the reference, since the claims measure the invention". *In re Lang*, 644 F.2d 856, 862, 209 USPQ 288, 293

(CCPA 1981). Moreover it is incumbent on Examiner to identify wherein each and every facet of the claimed invention is disclosed in the reference. *Lindemann Maschinen-fabrik Gmbh v. American Hoist and Derrick Co.*, 730 F.2d 1452, 221 USPQ 481 (Fed. Cir. 1984). Further, the reference must describe the invention as claimed sufficiently to have placed a person of ordinary skill in the art in possession of the invention. An inherent property has to flow naturally from what is taught in a reference *In re Oelrich*, 666 F.2d 578, 581, 212 USPQ 323, 326 (CCPA 1981).

**The rejected claims**

In this instance, all of the rejected claims are directed to methods in which conjugates bind to chemokine receptors on targeted cells. Il-2 binds to Il-2 receptors, which are not chemokine receptors. Volk *et al.* does not disclose a method for targeted delivery of an agent associated with a chemokine receptor targeting agent to cells. Therefore, as discussed in detail below, Volk does not expressly or inherently disclose a method in which a conjugate binds to chemokine receptors. Thus, Volk *et al.* cannot anticipate any of the claims.

To focus of the remarks herein, subject matter of the claims, particularly the independent claims is summarized:

Claim 35 is directed to methods of targeted delivery an agent into cells that express chemokine receptors by:

associating the agent with a chemokine receptor targeting agent, whereby:

the *chemokine receptor targeting agent binds to a chemokine receptor* expressed on the cells; and

the agent is internalized by the cells.

Claim 38 is directed to methods of inhibiting proliferation, migration or activation of cells bearing chemokine receptors by:

contacting the cells with an effective amount of a conjugate that comprises a targeted agent and a chemokine receptor targeting agent, or a portion thereof, wherein the *conjugate binds to a chemokine receptor* resulting in internalization of the targeted agent in cells bearing the receptor.

Claim 72 is directed to methods of inhibiting proliferation, migration or activation of immune cells by:

> contacting immune cells with a conjugate that comprises a targeted agent or a portion thereof and a chemokine receptor targeting agent whereby activation, proliferation, migration of the immune cells is inhibited, wherein:
>
> the targeted agent or portion thereof is a toxin;
>
> the chemokine receptor targeting agent is a chemokine or a fragment of thereof that binds to a chemokine receptor and internalizes the targeted agent; and
>
> *the conjugate binds to a chemokine receptor* resulting in internalization of the targeted agent in cells bearing the receptor.

Claim 86 is directed to methods for developing methods of treatment of inflammatory disorders:

> A method of treating a disease or disorder associated with an inflammatory response by:
>
> identifying immune cells that are activated in the disease or disorder;
>
> identifying chemokine receptors expressed on the cells;
>
> preparing a conjugate or plurality thereof containing toxin linked to a chemokine or a plurality of chemokines that specifically bind to the identified chemokine receptors and effect or facilitate internalization of the toxin into the cells; and
>
> contacting the immune cells with the conjugate or plurality thereof.

All claimed methods target conjugates to cells that express chemokine receptors.

**Differences between the disclosure of the cited reference and claimed subject matter**

**Volk *et al.***

Volk discloses a chimeric protein IL-2-PE40. IL-2 is not a chemokine nor is it a chemokine targeting agent since it does not bind to chemokine receptors; it binds to IL-2 receptors.

Volk *et al.* does not disclose any conjugates that bind to chemokine receptors nor (although not relevant to a rejection under 35 U.S.C. §102) suggest substitution of a chemokine receptor targeting agent for IL-2.

As noted, to anticipate a claim, a reference must disclose every element as claimed. The following claim-by-claim analysis (focusing on the independent claims) demonstrates that Volk *et al.* does not disclose every element as claimed, and, thus, does not anticipate any rejected claim.

**Claim 35 and dependent claims**

Claim 35 is directed to methods of targeted delivery an agent into cells that express chemokine receptors by:

**a)** associating the agent with a chemokine receptor targeting agent, whereby:

**b)** the *chemokine receptor targeting agent binds to a chemokine receptor* expressed on the cells; and

the agent is internalized by the cells.

**a)** "associating the agent with a chemokine receptor targeting agent"

Volk *et al.* does not disclose associating any agent with a chemokine receptor targeting agent. As described in the specification, a chemokine receptor targeting agent is an agent that binds to a chemokine receptor. For example, at page 30, the specification states:

> As used herein, a chemokine receptor targeting agent refers to any molecule or ligand that specifically binds to a chemokine receptor on a cell and effects internalization of a linked or otherwise associated targeted agent.

Further, as described in the specification (page 35), chemokine receptors are receptors refer to:

> receptors that specifically interact with a naturally-occurring member of the chemokine family of proteins and transport it into a cell bearing such receptors. These include, but are not limited to, the five receptors (CXCR1-5) to which CXC chemokines bind and the nine receptors (CCR1-9) to which CC chemokines bind, and any other receptors to which any chemokine will specifically bind and facilitate internalization of a linked targeted agent.

Il-2 binds to Il-2 receptors; there is no evidence cited by the Examiner nor of record demonstrating that IL-2, which is not a chemokine, binds to any chemokine receptors. Thus, Volk *et al.* does not disclose a method that includes a step of associating an agent with a chemokine receptor targeting agent.

**b)** the "chemokine receptor targeting agent binds to a chemokine receptor"

As discussed above, Il-2 is not a chemokine receptor agent nor does it bind to a chemokine receptor.

Thus, Volk *et al.* does not disclose a method that includes one or both of the elements of associating the agent with a chemokine receptor targeting agent and/or binding of the resulting conjugate to a chemokine receptor. Thus, Volk *et al.* does not disclose all elements (or any elements) of claim 35. Therefore Volk *et al.* does not anticipate claim 35 nor any claims dependent thereon.

**Claim 38**

Claim 38 is directed to methods of inhibiting proliferation, migration or activation of cells bearing chemokine receptors by:

> contacting the cells with an effective amount of a conjugate that comprises a targeted agent and a chemokine receptor targeting agent, or a portion thereof, wherein the *conjugate binds to a chemokine receptor* resulting in internalization of the targeted agent in cells bearing the receptor.

Claim 38 requires contacting cells with a conjugate that includes a targeted agent and a chemokine receptor targeting agent, where the conjugate binds to a chemokine receptor. As discussed above, there is evidence cited by the Examiner that IL-2 binds to chemokine receptors, therefore Volk *et al.* does not disclose all (or any elements) of claim 38. Thus, Volk *et al.* does not anticipate claim 38 or any claims dependent thereon.

Claim 72 is directed to methods of inhibiting proliferation, migration or activation of immune cells by:

> contacting immune cells with a conjugate that comprises a targeted agent or a portion thereof and a chemokine receptor targeting agent

whereby activation, proliferation, migration of the immune cells is inhibited, wherein:

the targeted agent or portion thereof is a toxin;

*the chemokine receptor targeting agent is a chemokine* or a fragment of thereof that binds to a chemokine receptor and internalizes the targeted agent; and

*the conjugate binds to a chemokine receptor* resulting in internalization of the targeted agent in cells bearing the receptor.

As discussed above, IL-2, which is not a chemokine nor does it bind to chemokine receptors, so it is not a chemokine receptor targeting agent. Thus, the conjugate of Volk *et al.* does not bind to chemokine receptor. Therefore, Volk *et al.* does not disclose all elements of claim 72 as claimed, and does not anticipate claim 72 or any dependents thereof.

Claim 86 is directed to methods for developing methods of treatment of inflammatory disorders:

A method of treating a disease or disorder associated with an inflammatory response by:

identifying immune cells that are activated in the disease or disorder;

*identifying chemokine receptors expressed on the cells;*

*preparing a conjugate or plurality thereof containing toxin linked to a chemokine* or a plurality of chemokines that specifically bind to the identified chemokine receptors and effect or facilitate internalization of the toxin into the cells; and

contacting the immune cells with the conjugate or plurality thereof.

Volk *et al.* does not disclose a method that includes a step of identifying chemokine receptors expressed on cells nor preparing a conjugate that contains a chemokine that specifically binds to the identified receptors. Thus, Volk *et al.* does not disclose all elements of claim 86 as claimed. Therefore Volk *et al.* does not anticipate claim 86 or any claims dependent thereon.

* * *

In view of the above amendments and remarks, reconsideration and allowance of the application are respectfully requested.

Respectfully submitted,
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# IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant: McDONALD et al.

Serial No.: 09/360,242

Filed: July 22, 1999

For: *METHODS AND COMPOSITIONS FOR TREATING SECONDARY TISSUE DAMAGE AND OTHER INFLAMMATORY CONDITIONS AND DISORDERS*

Art Unit: 1647

Examiner: Landsman, R.

I hereby certify that this paper and the attached papers are being deposited with the United States Postal Service as first class mail in an envelope addressed to:

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10/09/02
Date

Stephanie Seidman

## ATTACHMENTS TO RESPONSE

1. Marked-up claims pursuant to 37 C.F.R. §1.21

2. An unexecuted DECLARATION pursuant to 37 C.F.R. §1.132, with attachments.

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Stephanie Seidman

## MARKED-UP CLAIMS

Please amend claims 35 and 81 as follows:

35. (Amended) A method of targeted delivery of an agent into cells that express chemokine receptors, comprising associating the agent with a chemokine receptor targeting agent, whereby:

the chemokine receptor targeting agent binds to a chemokine receptor expressed on the cells; and

the agent is internalized by the cells.

81. (Amended) The method of claim [65] 35, wherein the chemokine is selected from the group consisting of IL-8, GCP-2, GRO-$\alpha$, GRO-$\beta$, GRP-$\gamma$, ENA-78, PBP, CTAP III, NAP-2, LAPF-4, MIG, PF4, IP-10, SDF-1$\alpha$, SDF-1$\beta$, SDF-2, MCP-1, MCP-2, MCP-3, MCP-4, MCP-5, MIP-1$\alpha$, MIP-1$\beta$, MIP-1$\gamma$, MIP-2, MIP-2$\alpha$, MIP-3$\alpha$, MIP-3$\beta$, MIP-4, MIP-5, MDC, HCC-1, LD78$\beta$, eotaxin-1, eotaxin-2, I-309, SCYA17, TARC, RANTES, DC-CK-1, lymphotactin, and fractalkine.